Glucagel,[1] A Gelling β-Glucan from Barley

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ABSTRACT

A new form of the mixed-linked (1→3),(1→4)-β-D-glucan has been obtained from barley grains using a new extraction and purification process that involves two key steps. A hot-water extraction of the β-glucan from the grain followed by a freeze and thaw of the extract. The commercial product from this process, called Glucagel, forms as a gelatinous or fibrous precipitate, which can be dried. Glucagel has novel functional properties. It forms soft thermoreversible, translucent gels that melt and set at temperatures of ≈60°C.

Mixed-linked (1→3),(1→4)-β-D-glucans are found as the major nonstarch polysaccharides in both oat and barley cereal grains. They generally have high molecular weights and form viscous solutions even at low concentrations. The main structural blocks are three and four β-(1→4) linked glucopyranosyl units (cellotriosyl and cellotetraosyl units) connected by single β-(1→3) linkages. Smaller amounts of structural blocks that contain more than four β-(1→4)-linked glucopyranosyl units are also present (Woodward et al 1983).

A high level of β-glucan in barley used for animal feed is considered undesirable because the high viscosity of the glucan impedes the adsorption of nutrients and therefore lessens the growth rate of animal. For humans however, foods, particularly oat-based foods, rich in β-glucan are considered to be beneficial because they lower serum cholesterol levels and flatten the glycaemic response to the digestion of starch (Wood 1994, Wursch and Pi-Sunyer 1997). Recently, the United States Food and Drug Administration has allowed claims that consumption of oat-based foods containing β-glucan reduces the risk of heart disease on the basis that it can lower serum cholesterol. Presumably, a similar claim could be allowed for barley-based products that contain β-glucan. For these reasons, there has been interest in obtaining cereal products enriched in β-glucan. Methods developed for enriching cereal foods in β-glucan have included milling and fractionating of cereal grains to obtain components rich in β-glucan (Knuckles et al 1992, Mälkki 1992, Wu and Stringfellow 1995, Wood et al 1991, Sundberg et al 1995), and extracting and purifying the β-glucan itself from the cereal grain (Wood et al 1989, Bhatty, 1995).

The extraction of β-glucan from cereal grains has generally involved three basic steps: deactivation of enzymes, extraction of the β-glucan, and then precipitation of the β-glucan. Enzymes are deactivated in the cereal grain before extraction because they are responsible for decreasing the molecular weight of the extracted β-glucan. They have usually been deactivated by refluxing the barley flour in aqueous ethanol or treating the barley flour with dilute aqueous acid. The β-glucan is then extracted with hot water (Saulnier 1994) or sodium carbonate solution (Wood et al 1989, Beer et al 1996). The water temperature is usually kept below 60°C to avoid starch gelatinization. The spent flour is removed by centrifugation, and the β-glucan is recovered from the supernatant by precipitation with either a water-miscible organic solvent or with ammonium sulfate solution. The precipitate, a gum, generally contains ≈40–60% β-glucan.

More recently, a new extraction procedure has been developed that has enabled more of the β-glucan to be recovered (Bhatty 1993, Bhatty 1995). Instead of hot water, 1.0*N* sodium hydroxide was used for the initial extraction, and the solids were removed by centrifugation. However, the procedure solubilizes considerable amounts of starch and protein. Starch was, therefore, hydrolyzed with Termamyl (Novo Nordisk, Copenhagen, Denmark) after the extract was neutralized. The protein was then precipitated by lowering the pH level. As in previous methods, a gum containing glucan was then obtained by precipitation with a water-miscible solvent, in this case ethanol. The gum was suspended in water to remove salts and other impurities. It was then washed and freeze-dried. Although it appears that the method of Bhatty does not contain an enzyme deactivation step, the extraction with 1.0*N* sodium hydroxide has the same effect.

The resultant products from these types of processes are gums that contain between 20 and 80% β-glucan. Generally gums with high purity are only obtained with additional enzyme processing steps that hydrolyze and solubilize protein and starch, during the extraction.

Current technology for the extraction and purification of β-glucan from cereal grains appears expensive because considerable amounts of organic solvents are required for the precipitation of the β-glucan once it is extracted into water. A significant number of processing steps are also required. The gum product formed has some undesirable properties; for example, it is difficult to dissolve. In this article we describe the development of a simple method for extracting and purifying a novel β-glucan product called Glucagel from barley.

MATERIALS AND METHODS

Preparation of Glucagel

Barley pollard flour (5 g) produced from a breeders selection on a Buhler mill (Buhler, Inc. Minneapolis, MN) was extracted with water (25 mL) at temperatures of 25–55°C for times of 0.5–6 hr. The spent flour from each fraction was centrifuged at 1,500 × *g* for 10 min, and the supernatant was frozen at –10°C for 24 hr. The frozen solution was then thawed in a water bath at room temperature. The gelatinous or fibrous material present in the thawed solution was recovered by filtration through a No. 2 sintered glass filter and washed with water (≈20 mL) and ethanol (≈20 mL) before being dried at 50°C. The resultant product, Glucagel, was purified by redissolving in water at 80°C, filtering through glass-fiber filter paper, and repeating the freeze-thaw step. The precipitate was filtered, washed with water (≈20 mL), and air-dried (or washed with 90% ethanol (≈20 mL) to remove additional water before being air-dried).

β-Glucan Content

The β-glucan content of the Glucagel was determined by the method of McCleary (McCleary and Codd 1991) using the Megazyme (Wicklow, Ireland) mixed-linkage β-glucan assay kit.

Molecular Weight Determination

Samples of the Glucagel were dissolved in 10 m*M* phosphate buffer (pH 6.9, 1% w/w) at 90°C. The molecular weight distribution

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was determined by gel-permeation chromatography (GPC) on a Superose 6 HR 10/30 column (AMRAD Pharmacia Biotech, Auckland, New Zealand) calibrated with Shodex pullulan standards P-82 (Phenomenex, Auckland, New Zealand) using 10 m*M* phosphate buffer as eluent at a flow rate of 0.5mL/min. The eluent was monitored by changes in refractive index. The standards had molecular weights (as measured by ultracentrifugal sedimentation equilibrium by the manufacturer) of 835,000, 380,000, 186,000, 100,000, 48,000, 23,700, 12,200, and 5,800. The molecular weight corresponds to the maximum of the chromatographic peak and is reported with respect to these pullulan values.

Differential Scanning Calorimetry (DSC)

Measurements were performed using a DSC 4207 (Hart Scientific, American Fork, UT). Samples for the DSC were prepared by dissolving purified Glucagel in water at 80°C to form a 5% solution and setting aside to cool for 12 hr before scanning. The nominal scan rate was 30°C/hr. The same sample was then left at room temperature for 12 hr before repeating the scan.

RESULTS AND DISCUSSION

The Glucagel process reported here consisted of two basic steps: extraction and freeze-thawing. There are, therefore, two key differences in the Glucagel process to those previously reported. First, there is no enzyme deactivation step; in fact, enzyme hydrolysis of the cell wall β-glucan and the solubilized β-glucan that is formed, is an integral and necessary part of the process. Second, the precipitation of the glucan was by freezing and then thawing of the extract, not by mixing the extract with a water-miscible organic solvent.

It is well known that enzymes are active during extraction of β-glucan from cereal grains. The precise origin of the enzymes and the functionality of the enzymes responsible for the hydrolysis in the Glucagel process has not been fully determined to date. It appears, however, that the enzymes are probably the so-called solubilases (Yin and MacGregor 1988, Yin and MacGregor 1989). These function differently from other β-glucan-degrading enzymes that are expressed during germination of the grain. Yin and MacGregor (1989) found that the solubilases appear to attack only β-(1→4)-linked blocks that are of DP ≈ 9 (Woodward et al 1983).

Whatever may be the action of the enzymes, enzyme hydrolysis occurs during extraction and results in a decrease in the molecular weight of the Glucagel with increasing extraction time. Typical results are shown in Table I for a barley pollard flour from a breeder's selection containing 6.8% β-glucan. For short extraction times of ≈0.5 hr and at temperatures of 55°C, the molecular weight of the product was reasonably high (≈560,000) with respect to the pullulan standards. With longer extraction periods of 24 hr, it was possible to obtain products with molecular weights as low as 14,000. At all extraction temperatures investigated, the molecular weight of the β-glucan decreased as the extraction time increased.

The amount of β-glucan extracted from the barley depended on extraction temperature. At 25°C, the amount of β-glucan extracted was 24% of the total β-glucan content of the flour, regardless of the length of the extraction process (Table I). With an extraction temperature of 40°C, the amount extracted increased to 30% of the total. At the highest extraction temperature investigated (55°C) and after 2 hr of extraction, the β-glucan extracted was 62% of the total. Thus, the extraction temperature was the main determinant of the yield of β-glucan, although at higher temperatures, there was some decrease in yield after extraction times of 3 hr (Table I). The amount of Glucagel that was extracted was probably significantly higher than this because, on average, ≈20% of the water added to the flour remained unrecovered from the barley flour after the centrifugation step. If it was assumed that this unrecovered water contained an equal amount of β-glucan, then the greatest amount of β-glucan extracted (at 55°C for 2.75 hr) was 74% of the total β-glucan content of the flour. Where analyzed, the β-glucan content of the Glucagel was ≈90 ± 5% (Table I).

The other crucial step in the Glucagel process, freezing and thawing, resulted in a precipitate that contained ≈80% water. For Glucagel of low molecular weight (≈50,000), the precipitate consisted of fine fibers, whereas for Glucagel of high molecular weight (≈560,000), the precipitate was more gelatinous and stringy in appearance. Additional freezing and thawing of the precipitate caused more water to separate out. Treatment with alcohol could be used to remove water as well. The Glucagel was purified by dissolving the impure material in water at temperatures >80°C and repeating the freeze-thaw process. This gave a product that had β-glucan content of 100 ± 5%.

Glucagel has interesting functional properties. Glucagel with a molecular weight <80,000 Da dissolved readily in water at concentrations as high as 20% (w/w) at temperatures >80°C. High molecular weight Glucagel dissolved less readily in water at temperatures >90°C to a concentration of ≈5% (w/w). On cooling <55°C, solutions containing >0.5% Glucagel set to form soft gels, regardless of molecular weight. The gels could be repeatedly melted over a temperature range of ≈55–75°C and formed again on cooling. DSC endotherms showed that the melting was surprisingly complex (Fig. 1). There was a large endotherm at 58°C, a smaller endotherm at 66°C, and also a broader endotherm at ≈75°C. If the gels were left to cool, then similar DSC were obtained on reheating. Thus the gels are thermoreversible.

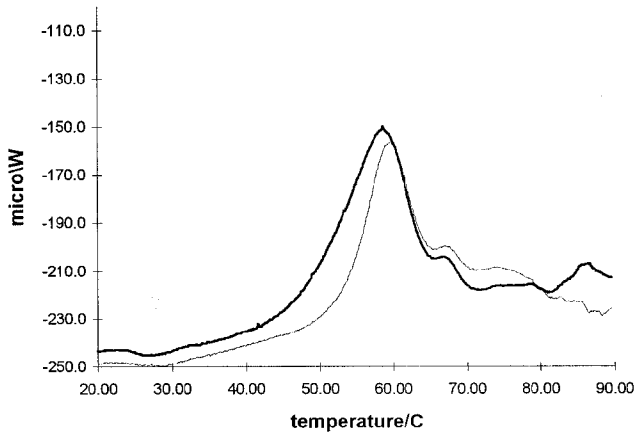

Fig. 1. Differential scanning calorimetry scans of a gel formed from 5% (w/w) purified Glucagel product in water. Second scan (pale line) was performed 12 hr after the first scan (dark line). Between scans sample was left at room temperature.

TABLE I
Glucagel Yields as a Percentage of the Weight of the Flour,[a] Percentage of β-Glucan Content, and Molecular Weights[b]

Extraction Temperature (°C)	Extraction Time (hr)	Glucagel Yield (%)	β-Glucan Content (%)	Molecular Weight at Peak Apex[c]
25	0.5	1.8	· · ·	62,000
25	2	1.9	94	53,000
25	3.5	1.6	92	46,000
25	5	1.6	· · ·	37,000
35	5	1.6	· · ·	30,000
40	0.5	2.5	82	79,000
45	2	2.8	90	49,000
45	5	1.9	91	31,000
55	2.75	4.7	90	62,000
55	5	3.6	94	56,000
55[d]	0.5	2.7	89	560,000

[a] Milled barley pollard flour produced from a breeders selection containing 6.8% β-glucan.
[b] With respect to pullulan standards.
[c] In gel-permeation chromatography.
[c] Pollard flour from the bran finisher.

Glucagel is a unique cereal β-glucan polysaccharide with novel functionality. Compared to other methods for isolating β-glucan from cereals the Glucagel process requires only a few steps, avoids the use of organic solvents, and results in a product of high purity. Glucagel could be obtained with a wide range of different molecular weights by simply controlling the extraction time. The ease with which Glucagel can be isolated should see many new uses develop for this novel cereal polysaccharide.

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